THE CHEMICAL PROPERTIES OF BACTERIAL GUM LEVAN.

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The production of gum levan by the action on sucrose of a new bacillus, *B. levaniformans*, isolated from samples of cane juice and of sugar which had been found to be undergoing inversion, is described by Mr. R. Greig-Smith, M.Sc., in the preceding paper. My examination into some of the properties of the gum was made on material given to me by Mr. Smith, for an abundant supply of which I am indebted to him.

The gum was purified from sugars by precipitation with alcohol from solution in water. After drying at 100° C. it contained 0·125 per cent. of nitrogen, equivalent to 1·0 per cent. proteid. The ash amounted to 1·4 per cent.

Deducting these constituents, which may be regarded as impurities, the preparation contained 97·6 per cent. of gum. It is on this figure that the products of inversion, &c., are calculated below.

The solution of the gum in water is opalescent, less so, however, when concentrated than when moderately dilute.

When a hot, strong solution is allowed to stand in the cold, it forms a mucilage resembling that produced by gum arabic, and does not become at all gelatinous.

The gum is insoluble in spirit, by which it is precipitated from aqueous solution.

It is not precipitated by subacetate of lead, nor by ammoniacal silver nitrate, but forms a white precipitate with ammoniacal lead acetate.

Owing to the persistent opalescence of the solution, which is not removed by any ordinary means of treatment such as filtration with hydrate of alumina, &c., it is not possible to determine the specific rotatory power of the gum except in dilute solution. A solution containing 1 gram. of the crude gum in 100 c.c. water observed in a 100 mm. tube in a Polarimètre-Laurent with monochromatic soda flame, gave a reading of $-0{\cdot}39°$ at 20° C., which is equivalent to a specific rotatory power of about $[a]_D^{20°} - 40°$ for the pure gum.

After oxidation at 60° C. with nitric acid of 1·24 sp. gr., oxalic acid was obtained, but neither mucic nor saccharic acids. The yield of oxalic acid varied in different trials between 12·8 and 16·8 $C_2 H_2 O_4$ per cent. on the pure gum, the solution also containing much reducing sugar. On treatment with dilute hydrochloric or sulphuric acid at 60 to 70° C., the gum is readily hydrolysed, the sole product being levulose, which is produced in practically the theoretical quantity required by the formula:—

$$C_6 H_{10} O_5 + H_2O = C_6 H_{12} O_6$$

In some respects this substance resembles Lippmann's levulan (Ber. 14, 1509), a body found in the juice of beetroots and in the residual molasses produced during the manufacture of sugar therefrom. The most important points of resemblance are in yielding levulose on hydrolysis, and in possessing a lævorotatory power.

From levulan it differs (*a*) in having a much lower rotatory power, that for levulan being, according to Lippmann, $[a]_D - 221°$, while the present substance is only about $[a]_D - 40°$; (*b*) in giving no blue precipitate with Fehling's copper solution; and (*c*) in the concentrated hot solution not gelatinising on cooling.

In his original description of levulan (*loc. cit.*), Lippmann stated that it yielded mucic acid on oxidation with nitric acid, and this statement has been copied into works of reference) Thorpe, Dictionary of Applied Chemistry, ii., 280, 1891; Watts's Dictionary of Chemistry, iii., 116, 1892; Allen's Commercial Organic Analysis, i., 423, 1898). Subsequently Lippmann (Ber. 25, 3216,

1892) corrected the above statement, he having found it to be erroneous.

The facts of the $[a]_D$ being $-40°$, and the gum yielding only levulose on hydrolysis at once suggested inulin, and I therefore made a careful comparison of its behaviour with sundry reagents, as against that of inulin, and, for purposes of comparison, the closely allied body starch.

The results of this comparative examination are stated in tabular form below.

	Gum Levan.	Inulin.	Starch.
$[a]_D$	Lævo.	Lævo.	Dextro.
Oxidation nitric acid ...	Oxalic acid. No mucic acid.	Oxalic acid. No mucic acid.	Oxalic acid. No mucic acid.
Cold water	Very soluble.	Sparingly soluble	Insoluble.
Hot water solution after cooling	Mucilaginous.	Limpid.	Gelatinous.
Heated for some time at 100°C. with water	Unchanged.	Hydrolysed to levulose.	Unchanged.
Hydrolysis with acid ...	Levulose.	Levulose.	Dextrose, &c.
Baryta water	Ppt.	Ppt.	Ppt.
Lime water	No ppt.	No ppt.	Ppt.
Cream of lime	Pptd.	Not pptd.	Pptd.
Strontia water	Ppt.	*	Ppt.
Lead subacetate	No ppt.	No ppt.	Ppt.
Ammonia lead acetate ...	Ppt.	Ppt.	Ppt.
Action of solution in cold on litharge	Does not dissolve litharge.	Dissolves litharge.	Does not dissolve litharge.
Ammonia silver nitrate in dark	No reduction.	Reduced.	No reduction.
Boiling Fehling copper solution	No reduction.	Slowly reduced.	No reduction.
Iodine	No action.	No action.	Blue compound.
Cuprammonium	Dissolves, does not deposit on standing.	Dissolves, throws down heavy ppt. on standing.	Does not perfectly dissolve. Throws down moderate blue sediment.

* Several examples of carefully purified inulin which I prepared from dahlia tubers grown at Sydney, gave a copious precipitate when a solution was mixed with strontia water, but a sample procured from Europe, the source of which I do not know, gave no reaction when treated in the same way, though all the other tests gave results identical with the Sydney preparations.

From a consideration of the foregoing it will be seen that gum levan differs from inulin in its properties about as much as the latter does from starch. The most striking points of resemblance between levan and inulin are the $[a]_D$ and the production, on hydrolysis, of levulose only.

The figures following show the effect of the growth of the bacillus in a solution of sucrose of 10 per cent. strength.

After removal of the gum by precipitation with alcohol, and concentration of the saccharine solution, the residual syrup obtained was found to contain for each 100 parts of sucrose originally present:—

Sucrose	5·4
Dextrose	41·4
Levulose	20·6

It is thus seen that during its growth the bacillus has inverted the sucrose, and then assimilated levulose. As sucrose, on inversion, yields equal proportions of dextrose and levulose, the difference between these two sugars in the residual syrup represents the levulose which has been used by the bacillus, and amounts to 20·8 per 100 original sucrose. If there were as much levulose present as dextrose, the sum of the two would be 82·8, which is equivalent to 78·7 of sucrose. This proportion, with the 5·4 still present, leaves 15·9 of sucrose not accounted for. A little of this will be due to mechanical losses during manipulation, and the remainder, which may be stated as at least 10 per cent., represents sucrose used up by the bacillus in addition to the missing 20·8 of levulose. These results were checked by the examination of several different cultures, and in each case results of a similar nature were obtained.

In a culture made in 20 per cent. solution of sucrose, the growth of the bacillus was not nearly so abundant as in those made in 10 per cent. solutions, and the transformation of sucrose correspondingly less complete.

In the disease known as "gumming" in sugar cane, on cutting the stalks across the fibro-vascular bundles are found to be

packed with a viscid gum. This substance is quite distinct from gum levan, and has properties differing considerably.

The above investigation was carried out in the laboratory of the Colonial Sugar Refining Company, Limited, Sydney, and I am enabled to make use of the results by kind permission of Mr. E. W. Knox, general manager, and Mr. T. U. Walton, B.Sc., F.I.C., &c., principal chemist.